

Long axons within the striate cortex: Their distribution, orientation, and patterns of connection

(horseradish peroxidase/stripes/orientation columns/tree shrew/cat)

GRAEME MITCHISON AND FRANCIS CRICK

The Salk Institute, Post Office Box 85800, San Diego, California 92138

Contributed by Francis Crick, March 8, 1982

ABSTRACT Rockland and Lund [Rockland, K. S. & Lund, J. S. (1982) *Science* 215, 1532–1534] have recently observed that an injection of horseradish peroxidase into the striate cortex of the tree shrew produces a patchy distribution of label adjacent to the injection site. They proposed that this pattern might be due to populations of neurons with long-range cortico-cortical connections that are interspersed with populations having no such connections. We suggest here an alternative explanation. We can account for the pattern by supposing that the label is carried by a system of oriented axons. We suppose that these axons link cells with similar orientation preferences and make their connections within a narrow strip of cortex whose direction is related to the orientation of the cells in question. We suggest that such connections could be involved in generating complex receptive fields from simple ones. Other possibilities are that they are used to generate very elongated receptive fields, inhibitory flanks, or end-stopping. We suggest a number of experimental tests of these ideas.

In a recent paper, Rockland and Lund (1) made the remarkable observation that a local injection of horseradish peroxidase (HRP) in the primary visual cortex (also called the striate cortex or area 17) of the tree shrew gives rise to labeling not only in the immediate neighborhood of the injection but also in small patches all around the injection site. Their tentative interpretation was that there may be two intercalated systems of interconnections in area 17, one with long-range connections that produced the patches of HRP labeling, and a second that lacks them. We show here that their pattern could be explained in a different way, by supposing that neurons which have similar orientation preferences are joined together by a system of oriented axons. We shall see that this hypothesis could explain in a natural way the construction of complex receptive fields from simple ones and of long oriented receptive fields from shorter ones.

When reconstructed from tangential sections, the HRP-labeled pattern forms a series of stripes. Humphrey *et al.* (2, 3) have shown that cells of a given orientation preference are arranged in a pattern of roughly parallel stripes, about 0.5 mm apart, in area 17 of the tree shrew. Rockland and Lund commented on the fact that the pattern they have observed closely resembles these orientation stripes, and they suggested that there might be a functional relationship between the two patterns. Let us consider what this might be.

Possible Explanations. The HRP pattern presumably reflects the connections of cells within the site of the HRP injection (we consider this in more detail later). Suppose a cell connects to all other cells nearby that have a similar orientation preference. Because the injection sites are considerably larger than the spacing between orientation columns and are likely to in-

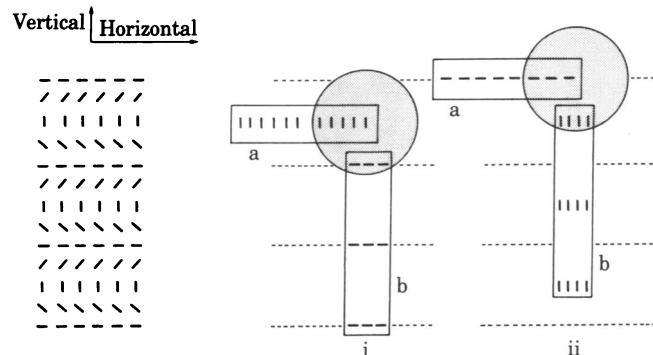


FIG. 1. An idealized set of orientation stripes is sketched on the left, the stripes following what corresponds to the horizontal direction in visual space. The stripes are assumed to continue running through the two adjacent sketches, with the horizontal stripe lightly indicated. Two separate cases are illustrated. In each the HRP injection is represented by a stippled circle. In the first case (i) we follow the convention that cells with similar orientations are joined within a narrow field whose direction is at right angles to the cells' orientation. Cells within the injection site having a vertical orientation are joined by a horizontal field (a) to others nearby, to give a continuous strip of label. Horizontally oriented cells connect within a vertical field (b) to patches of cells. The second case (ii) employs the other convention, that cells connect within a field of like orientation. We see that now it is the horizontally oriented cells that are joined to others within a continuous strip (a). The vertical cells connect to patches of cells (b).

clude cells of all orientations in roughly equal proportions, it is clear that, if all cells of similar orientation were interconnected, one would get a fairly uniform pattern of HRP, radiating away from the injection site. So this cannot account for the patchiness of the HRP pattern.

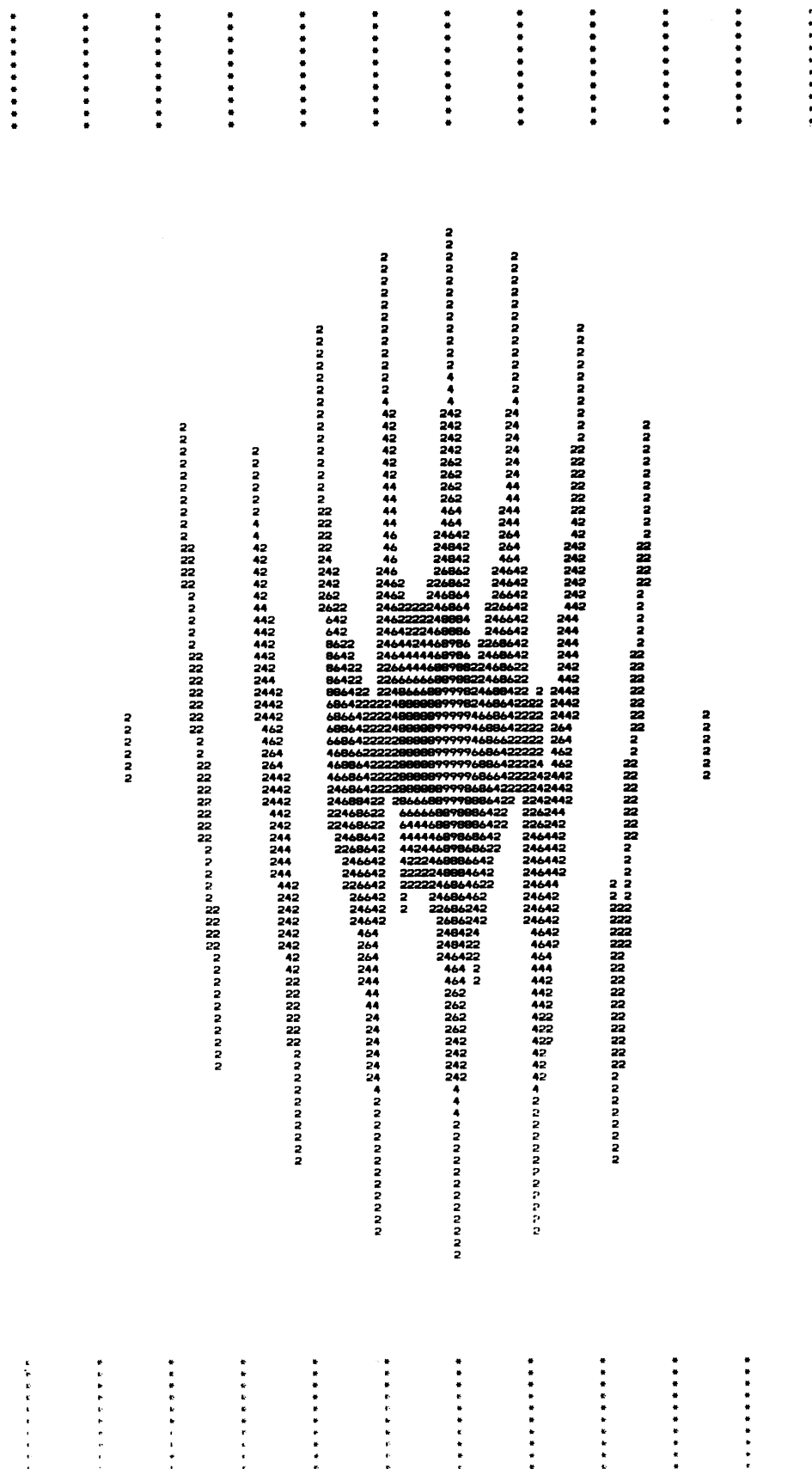
At this point, it is reasonable to ask what purpose connections between cells with similar orientations might serve. Now, amongst cells in the striate cortex, one can broadly distinguish two types of receptive fields (4). One, the so-called simple type, gives a response to oriented bars at a particular location in space; the other, the complex type, responds to an oriented bar over a range of positions in space. Hubel and Wiesel (4) have suggested that complex fields could be made by joining together a number of simple fields side by side. To do this, one would need to join cells with like orientations. But instead of joining *all* such cells within some distance, one would generally need to join only those that lie within a strip running in a particular direction on the cortex.

To see this, observe that at any point in the visual field a particular direction can be mapped onto the cortex as a direction in the tangential plane of the cortex. This is because, small distortions aside, the striate cortex is a map of the visual field. So, to construct a complex field by joining simple ones side by side,

the connections would have to be made in a direction at right angles to the cell's orientation preference, mapped onto the cortical surface.

Another possible role for the cortical connections we are con-

sidering would be in constructing very elongated fields by joining shorter ones end to end. A cell with such a field would then receive axon collaterals from distant cells having shorter receptive fields. But here the axons would have to follow a direction



roughly parallel to the cell's orientation preference, instead of at right angles, as for complex cells.

Our proposal is, then, that the HRP pattern is created by axons joining cells of like orientations in a particular direction on the cortex. The relationship between this direction and the cell's orientation preference will depend upon the type of field being constructed, complex fields giving one rule and elongated simple cells another.

The Stripes Illustrated. These two possible rules are illustrated in a highly idealized way in Fig. 1. In this figure the orientation stripes have been drawn to run horizontally. As one moves vertically in the figure, across the stripes, the orientations rotate steadily in a fixed direction. In the tree shrew the

stripes are about 0.5 mm apart and, near the 17/18 border, are arranged roughly perpendicular to this border. (The 17/18 border is the representation of the vertical meridian in the retinotopic map.)

The first possible rule is illustrated in case *i* of Fig. 1. Consider for the moment only cells with a *vertical* orientation preference (field a). We join up only such cells that lie in a horizontal direction in the cortex from the HRP injection. These can be seen to lie along an orientation stripe, so all the cells in this stripe will be joined. Now consider only those cells within the HRP injection site that have a *horizontal* preference (field b). If these are connected only with other similar cells that lie in a vertical direction in the cortex from the HRP patch, they do

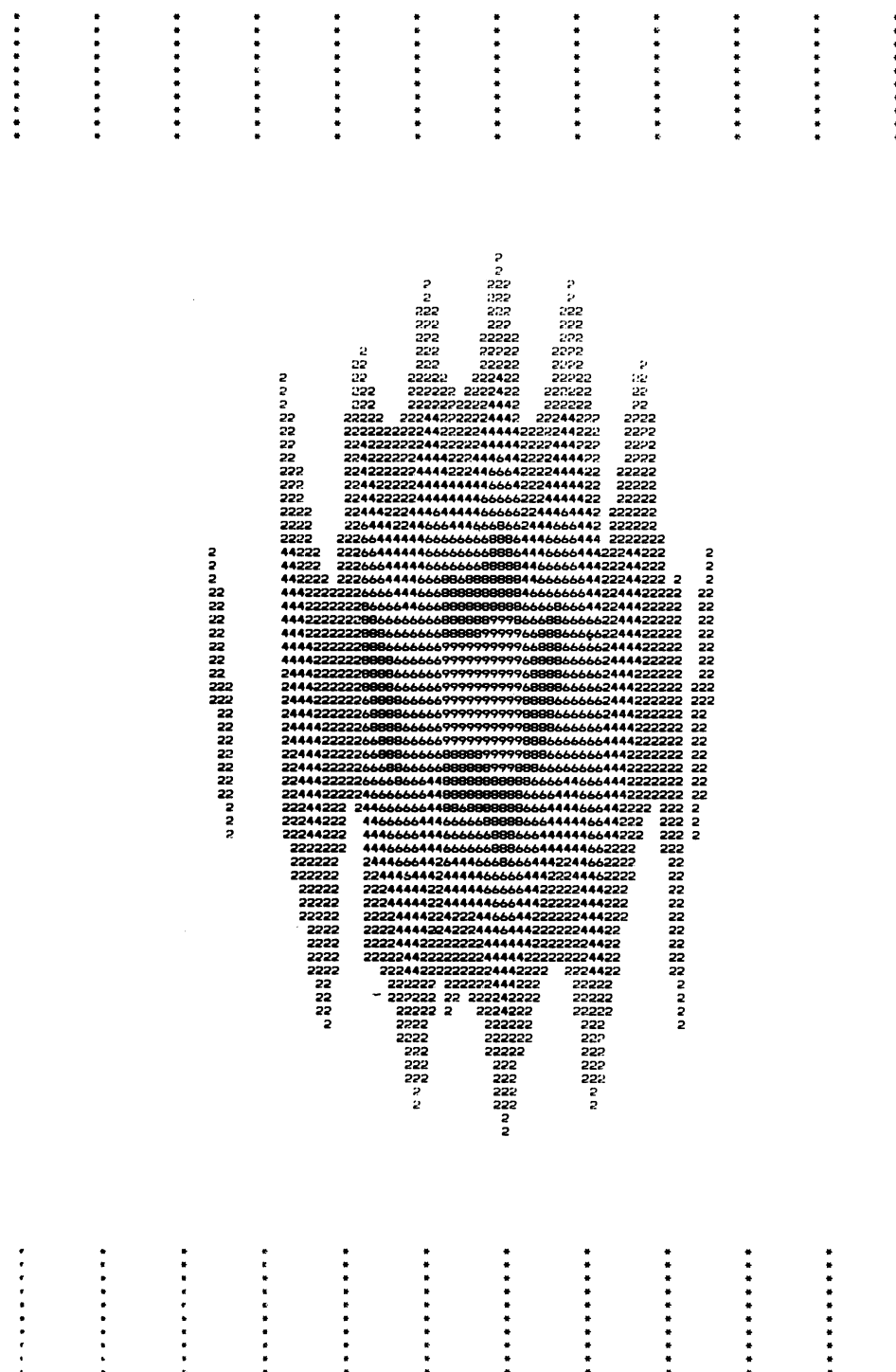


FIG. 2. Computer simulation of the stripes generated by using the rules illustrated in Fig. 1. Orientations were represented as parallel stripes on a regular grid, with a complete (180°) rotation in 10 steps on the grid. The orientation stripes run from the top to the bottom of the figure, as suggested by the short line segments above and below. If we adopt convention i of Fig. 1, then the lines drawn correspond to the vertical orientation; if we adopt convention ii , they are horizontal. We gave the connection field the shape of a Gaussian $G(u,v) = \exp[-(u^2/2a^2) - (v^2/2b^2)]$, in which the field is assumed to have its long axis in the u direction and its short axis in the v direction. The HRP injection site was represented by a 10×10 square of grid points, each point given a HRP concentration of c . Suppose the grid has coordinates (x, y) , with the y coordinate parallel to the orientation stripes. If the point P with coordinates (x, y) in the grid had the same orientation as a point $Q_i = (x_i, y_i)$ in the injection site, then a HRP concentration $cG(x - x_i, y - y_i)$ was added to P . Such contributions were summed over all Q_i with the same orientation within the injection site. In these calculations we took $c = 1$, which meant that the HRP concentrations at each point lay in the range 0–10, the integral parts of which are shown by the digits in the printout. (Zero has been omitted and only the even numbers have been shown, except for 9.) (*Left*) The variance a was taken to be 20 steps, and b to be 5 steps. Because the repeat distance between stripes is 10 units, these correspond to distances of 2 column widths by 0.5 column width. (*Right*) Here we took $a = 15$, $b = 10$, corresponding to 1.5 by 1 column widths.

not form a continuous strip, because they cut across the orientation stripes. (We imagine that in such cases the axon collaterals run without interruption and ramify extensively only when they come to a group of cells that have the correct orientation preference. Only the regions where the axons are highly ramified will show up in the HRP pattern.)

If we now fill in the pattern by adding connections from cells of *all* orientations (always using the rule that the connections are made only in a direction of the cortex perpendicular to the orientation preference in question) it is easy to see that all these patches join up into a fairly continuous system of stripes.

The second rule is illustrated in case *ii* of Fig. 1. In this case the rule is that the straight connection lines are *parallel* to the orientation preference (mapped onto the cortex) of the cells that are joined together. By considering each orientation preference in turn we see that the HRP pattern will again be a pattern of stripes, though these stripes will interleave with the stripes formed in the first case considered. In both cases the stripes will be roughly parallel to the orientation stripes.

The general nature of the stripes can be seen rather more clearly in the computer simulation shown in Fig. 2. In this figure the idealized orientation stripes have been drawn vertically rather than horizontally as they were in Fig. 1. It has been computed with reasonable rules for the fall-off of connections with distance, which also allow for a certain scatter in the direction of connection. The connection rule is more diffusely oriented in Fig. 2 *Right* than it is in Fig. 2 *Left*; the details are given in the legend to the figure. It can be seen that in both patterns, but especially in Fig. 2 *Left*, the calculated stripes roughly follow the orientation stripes (which are marked only at the top and bottom of the figure to avoid confusion). They are usually not strictly parallel to them but are inclined at a small angle. Put in other words, the computed "HRP stripes" follow the orientation stripes but with a phase shift that depends on the angle joining a given point to the HRP injection site.

Complications. The orientation stripes in the tree shrew are not nearly as regular as the idealized grid we have used. They appear to consist of fairly large areas of nearly parallel stripes, interrupted by singularities [see figure 10 of Humphrey *et al.* (3)]. Moreover, during a long electrode penetration in a direction at right angles to the orientation stripes, the orientations encountered may steadily change in one direction up to a certain point, and then proceed to change in the opposite sense. Both singularities and changes in rotation would be expected to disturb the pattern. Singularities will introduce blind endings or branches, and HRP stripes will run together along a boundary where the rotation sense changes (Fig. 3). All one can say in general is that the HRP pattern will run roughly parallel to the orientation stripes, perhaps showing branches or anastomoses. Rockland and Lund's patterns fit this general description, having a similar spacing and direction to the orientation stripes, with some branches.

We have used here a very crude definition of connectivity. Rockland and Lund found that HRP was transported in an orthograde manner along the axons of cells whose bodies lay in the injection site. They also found a small number of retrogradely filled cell bodies. If HRP is carried to the body of such a cell along one axonal branch and is then carried further in the same direction along another axon branch, the label could move greater distances from the injection site. However, one might expect that only a small amount of label moves in this way.

Rockland and Lund's pattern was found only in the superficial layers (II and III), the distribution being continuous in the deeper layers. The most likely candidates for the HRP-filled cells are small pyramids in layers II and III, whose axons travel distances of 1 mm or more in the upper layers. The size of the

oriented connection field we have used is therefore reasonable, but our calculation does not allow for retrograde movement to the cell body and beyond, which, if appreciable, would spread the stripes yet wider.

The Cat and the Tree Shrew. To interpret the system of connections for the tree shrew, we consider first what is known for another animal, the cat. As Gilbert (5) has shown, the cells in layers II and III in area 17 of the cat have receptive field widths very similar to those found in layer IV, which is the layer that receives most of the input from the lateral geniculate nucleus. The principal difference between cells in layers II and III and those in layer IV is that many of the former have complex receptive fields, whereas the latter are mostly simple. Gilbert (5) found that the length of bars that provided the optimal stimulus for complex cells in layers II and III was about the same as that for the simple cells in layer IV. He therefore suggested, following a proposal by Hubel and Wiesel (4), that a side-by-side joining-up operation is the main transformation that occurs in going from layer IV up to the supragranular layers. If the pattern of intracortical wiring in the tree shrew is similar to that in the cat (which there is every reason to believe is the case), we propose that the axon system shown up by the HRP pattern in the tree shrew carries out a similar operation in the upper layers of the cortex, and it consists of axons running at right angles to the preferred orientations of both their cells of origin and of the cells with which they connect.

The cat appears to have a system of orientation stripes like that in the tree shrew (6), so we might expect to find a pattern like Rockland and Lund's in the upper layers of the cat's striate cortex. However, we might also look for another type of connectivity. Gilbert and Wiesel (7) recorded from cells in area 17 of the cat, and then filled them with HRP. They described a long axon from a layer V cell that traveled a great distance through layer VI, and they noticed that the orientation of the axon in the cortex paralleled the preferred orientation of its parent cell. They conjectured that this arrangement could be involved in making the very long receptive fields that are characteristic of many layer VI cells. So, instead of joining oriented fields side-by-side to make complex fields, we should also consider that short fields may be strung end-to-end. In this case, we should expect to find that a HRP injection gave a stripe-like

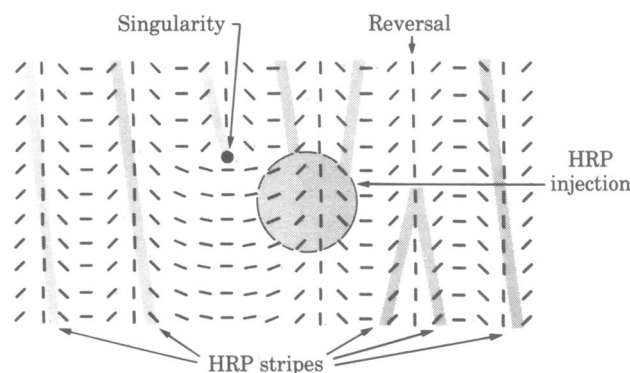


FIG. 3. The types of HRP pattern expected, using convention *i* of Fig. 1, when the orientation pattern is a little more complicated than the idealized one used before. In this figure the orientation stripes run from top to bottom. The HRP injection site is represented by the shaded circle. The HRP stripes (also shaded) were estimated, not calculated. On the left a singularity, *S*, is shown, of the kind postulated in figure 10 of Humphrey *et al.* (3). On the right there is a simple reversal so that the rate of change of orientation, going from left to right, changes sign at this point. As can be seen, the pattern of HRP stripes is distorted in these cases. Other cases, such as inclined reversals, give even more complicated distortions. Nevertheless, the general character and trend of the orientation stripes and the HRP stripes are similar.

pattern, but with the stripes shifted through one half-period of the underlying orientation pattern, because the cortical connections are turned through a right angle (Fig. 1, case ii, a and b). This pattern might be seen in the lower layers of the cat's striate cortex.

We should not expect to find a pattern of this kind in the lower layers of the tree shrew cortex, however, because it appears to lack cells with the very elongated receptive fields of the type found in layer VI of the cat. In fact, cells in the lower layers of the tree shrew's area 17 are less oriented than those in the supragranular layers (2). This may explain why Rockland and Lund's pattern shows no patchiness in layers V and VI. The computed HRP pattern for cells with less elongated fields has less conspicuous stripes (Fig. 2 *Right*).

Other Possibilities. We have now considered two ways in which systems of oriented axons might arise in the striate cortex. Another possibility is that there are axons leading to or from inhibitory cells, creating either the inhibitory flanks of receptive fields or the end-stopping that, in the cat, is common in layer IV and above (7). The former would yield a pattern similar

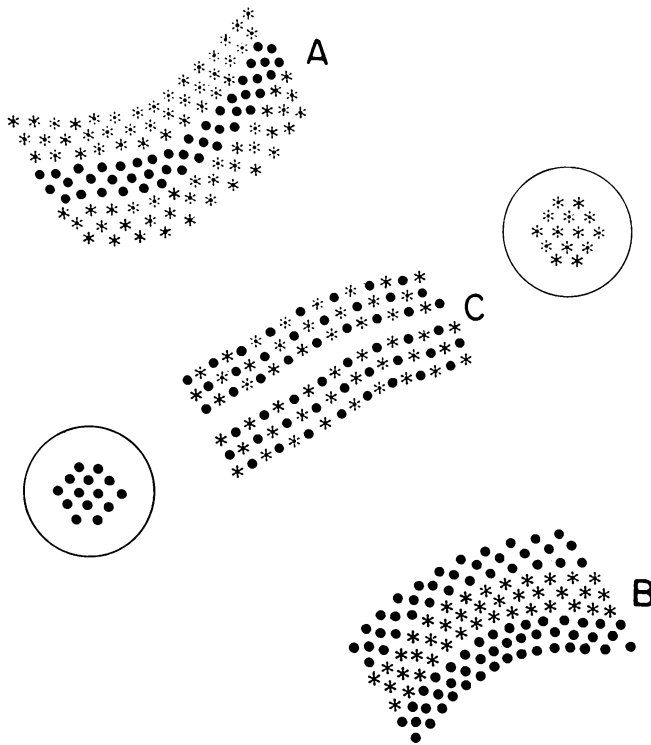


FIG. 4. Two injection sites, each injected with a different tracer, are represented by the two large circles. For simplicity the expected HRP stripes are shown only in the regions labeled A, B, and C. To distinguish the contributions from each site to neighboring cells, we label one with small circles, the other with asterisks. At a point C on the line joining the two sites, the connection field joining a cell to one of the sites has the same orientation as the field joining that cell to the other site. So any cell that is labeled by one site will be labeled (even if only weakly) by the other site, and the stripes of label will superpose (circles and asterisks intermingled). At either A or B, a cell that connects to one site cannot connect to the other, because the two connection fields would be at 90° to each other. Instead, each site will label interleaving stripes that differ by approximately 90° in orientation (circles and asterisks filling in adjacent bands). This argument holds no matter where the two injections are made, provided the retinotopic map is not grossly distorted on the large scale.

to the one proposed for making complex fields, the latter would create the 90° phase-shifted version. We have thus considered four ways, excitatory and inhibitory, in which the appropriate kinds of connections might arise. Which of these, if any, is involved in generating the HRP pattern remains to be seen. But it should perhaps be pointed out that if two such mechanisms operate at once, their combined effect might either enhance or conceal the HRP stripes, depending whether they are in phase or 90° out of phase.

Conclusions. If our general hypothesis is correct, then axons in a tangential section, prepared as in Rockland and Lund's experiments, should be oriented and be connected to other cells whose axons have the same orientation preference. If axons are involved in creating complex fields, or flank inhibition, the direction of a cell's axon should correspond to an orientation in visual space that is at right angles to the preferred orientation of the cell. If axons are used to generate elongated fields, or end-stopping, then the axons' direction should coincide with the preferred orientation of the cells.

In either case, the HRP stripes should run roughly parallel to the orientation stripes, with perturbations near to singularities, or boundaries where there is a reversal of the direction of change of orientation (Fig. 3). There should be a variable phase shift between orientation and HRP stripes, depending upon the direction from a given point to the injection site. To illustrate this, suppose two injections of different tracer molecules (HRP and some other tracer transported in a similar manner) were made into the striate cortex, we should expect that on a line joining the two injection sites (e.g., region C in Fig. 4), stripes of label from the two sites would superpose. But near those points at which the sites subtend a right angle, (regions A and B in Fig. 4), the stripes should be 90° out of phase and interleaved.

In summary, there appear to be three main experimental questions: (i) Is the pattern found by Rockland and Lund due to stripes of cells with long collaterals interleaved with stripes containing no short collaterals? Or, as we suggest here, do all regions of area 17 have long collaterals but only in particular directions from any one small patch of cortex? (ii) If the latter is correct, what is the relationship between the orientation preference of a cell and the orientation of its collaterals? (iii) What is the functional significance of these long connections?

Naturally the answers to these questions may differ from species to species and from one cortical layer to another. However, in all cases it seems likely that the questions can be answered with present experimental methods.

We thank Drs. Kathleen Rockland and Jennifer Lund for sending us their paper in advance of publication. We also thank them and Dr. Maxwell Cowan for many helpful comments on various drafts of this paper. This work was supported by the Samuel Roberts Noble Foundation, the J. W. Kieckhefer Foundation, the Eugene and Estelle Ferkhauf Foundation, and U.S. Air Force Grant AF OSR-82-0042.

1. Rockland, K. S. & Lund, J. S. (1982) *Science* **215**, 1532-1534.
2. Humphrey, A. L. & Norton, T. T. (1980) *J. Comp. Neurol.* **192**, 531-547.
3. Humphrey, A. L., Skeen, L. C. & Norton, T. T. (1980) *J. Comp. Neurol.* **192**, 549-566.
4. Hubel, D. H. & Wiesel, T. N. (1962) *J. Physiol. (London)* **160**, 106-154.
5. Gilbert, C. D. (1977) *J. Physiol. (London)* **268**, 291-421.
6. Singer, W., Freeman, B. & Rauschecker, J. (1981) *Exp. Brain Res.* **41**, 199-215.
7. Gilbert, C. D. & Wiesel, T. N. (1979) *Nature (London)* **280**, 120-125.